Critical Review

Fumonisins Disrupt Sphingolipid Metabolism, Folate Transport, and Neural Tube Development in Embryo Culture and In Vivo: A Potential Risk Factor for Human Neural Tube Defects among Populations Consuming Fumonisin-Contaminated Maize[1]

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ABSTRACT Fumonisins are a family of toxic and carcinogenic mycotoxins produced by *Fusarium verticillioides* (formerly *Fusarium moniliforme*), a common fungal contaminant of maize. Fumonisins inhibit ceramide synthase, causing accumulation of bioactive intermediates of sphingolipid metabolism (sphinganine and other sphingoid bases and derivatives) as well as depletion of complex sphingolipids, which interferes with the function of some membrane proteins, including the folate-binding protein (human folate receptor α). Fumonisin causes neural tube and craniofacial defects in mouse embryos in culture. Many of these effects are prevented by supplemental folic acid. Recent studies in LMBc mice found that fumonisin exposure in utero increases the frequency of developmental defects and administration of folate or a complex sphingolipid is preventive. High incidences of neural tube defects (NTD) occur in some regions of the world where substantial consumption of fumonisins has been documented or plausibly suggested (Guatemala, South Africa, and China); furthermore, a recent study of NTD in border counties of Texas found a significant association between NTD and consumption of tortillas during the first trimester. Hence, we propose that fumonisins are potential risk factors for NTD, craniofacial anomalies, and other birth defects arising from neural crest cells because of their apparent interference with folate utilization. J. Nutr. 134: 711–716, 2004.

KEY WORDS: • *fumonisins* • *neural tube defects* • *craniofacial abnormalities* • *sphingolipids* • *folate*

Fumonisins are a family of mycotoxins that were first isolated in South Africa in 1988 from cultures of *Fusarium verticillioides* (formerly *Fusarium moniliforme)* strain MRC 826 (1), followed soon thereafter by elucidation of the structures of the prevalent isoforms fumonisin B_1 (FB_1)[3] and B_2 (FB_2) (2). Leukoencephalomalacia in horses and pulmonary edema syndrome in pigs (3) were shown to result from administration of FB_1 (4,5), and field outbreaks were associated with fumonisin contamination (6) when analytical methods were developed (7). Fumonisins were also implicated in esophageal cancer when they were found in home-grown maize in a high-inci-

[1] Supported in part by the March of Dimes, the Nebraska Research Initiative, the South African Medical Research Council (PROMEC), the U.S. Department of Agriculture, the U.S. National Institutes of Health (GM46368), and the Centers for Disease Control (U85/CCU608761–05 and U50/CCU613232). Support for the workshop on "Fumonisins in Neural Tube Defects" held at Georgia Tech on January 28, 2003, was from the endowment for the Smithgall Institute Chair in Molecular Cell Biology and the U.S. Centers for Disease Control and Prevention.

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[3] Abbreviations used: AP_1, aminopentol or hydrolyzed fumonisin B_1; FB_1, fumonisin B_1; FB_2, fumonisin B_2; FB_3, fumonisin B_3; NTD, neural tube defects; OR, odds ratio.

Manuscript received 17 December 2003. Initial review completed 28 December 2003. Revision accepted 28 December 2003.

dence area of the Transkei region of South Africa (8). FB_1 has been demonstrated to cause liver and kidney cancer in rats and mice (9,10), with differences in species and sex (10), and the International Agency for Research on Cancer evaluated FB_1 as a Group 2B carcinogen, i.e., possibly carcinogenic to humans (11). In 2002 the Joint FAO/WHO Expert Committee on Food Additives allocated a group provisional maximum tolerable daily intake of 2 μg/kg body weight to FB_1, FB_2, and FB_3, alone or in combination (12).

In addition to the known toxicity and carcinogenicity of fumonisins, evidence has begun to surface that these mycotoxins can also be teratogenic, at least in part through interference with the utilization of folic acid, a dietary supplement used to reduce the incidence of neural tube defects (NTD).[4] The current knowledge about fumonisins as possible risk factors for birth defects was recently collated at the "Workshop on the Role of Fumonisins in Neural Tube Defects" in Atlanta, Georgia, on January 21, 2003, and this information is summarized in this review.

Disruption of sphingolipid metabolism

The mechanism(s) of action of fumonisins appears to involve disruption of sphingolipid biosynthesis by the inhibition of ceramide synthase (13–15), modification of cellular proliferation through changes in cell cycle regulators (16–19), and increased expression of cytokines such as TNFα (19–21). Fumonisins inhibit ceramide synthase because they have structural features that resemble the cosubstrates (sphingoid bases and fatty acyl-CoA's) (14,22), and inhibition has been characterized in vitro as well as in numerous cell lines, animals, plants, and fungi (14,15). Sphinganine accumulates rapidly in vivo and provides a biomarker for fumonisin exposure that has been validated in cells in culture, tissues, serum, and urine (23–25). Depending on the system studied, other metabolites are also elevated (sphingosine, sphinganine 1-phosphate, and *N*-acetyl-sphinganine and -sphingosine) (26) and significant depletion of complex sphingolipids (ceramide, sphingomyelin, and glycosphingolipids) can occur (13–15).

Because sphingolipids are involved in diverse aspects of cell regulation, disruption of sphingolipid metabolism may underlie many of the aforementioned mechanisms for the toxicity and carcinogenicity of fumonisins. Free sphingoid bases are growth inhibitory and cytotoxic (27) for cells in culture and blockage of sphinganine accumulation reduces the toxicity of FB_1 (28). Sphinganine accumulation in vivo is closely related to liver and kidney toxicity (14,29–32), with increases in free sphinganine usually occurring at or below the fumonisin dosages that cause liver or kidney lesions in rats (32), rabbits (33), mice (34), horses (23), pigs (24), and many other species (15,35) as well as nonneoplastic and neoplastic kidney lesions in Fischer-344/N Nctr BR rats (10). The appearance of free sphinganine in urine is most likely a result of a loss of cell anchorage and detachment into the kidney tubule lumen (36) because most free sphinganine is recovered in dead cells (31). The elevation in urinary sphinganine is reversible and subsides soon after the complete removal of fumonisins (although a supposedly subtoxic dose will maintain elevated sphinganine in rats and mice following exposure to a higher dose) (14,37,38). Extrapolation of these findings to humans is difficult, but disruption of sphingolipid metabolism has been associated with liver and kidney toxicity in nonhuman primates exposed to fumonisins (39). A recent study in China found that the free sphinganine to free sphingosine ratio was significantly greater in urine of males from households where the estimated daily FB_1 intake was >110 μg/kg body weight per day (40).

Inhibition of folate transport

In the early 1990s, following investigation of an NTD cluster that occurred among Mexican-American women in Cameron County, Texas, Kate Hendricks from the Texas Department of Health began questioning researchers about the possibility that exposure to fumonisin-contaminated food (tortillas) during gestation may have contributed to these birth defects [reviewed in (41)]. NTD are common congenital malformations that occur when the embryonic neural tube, which ultimately forms the brain and spinal cord, fails to properly close during the first few weeks of development. NTD are among the most common of all human birth defects, yet their etiologic basis and embryology remain poorly understood. Empirical risk figures, along with numerous clinical studies, indicate that NTD are of a multifactorial origin, having both genetic and environmental components (41–47). Epidemiological studies indicate that periconceptional vitamin supplements containing folic acid can significantly reduce (50–70%) a woman's risk for an NTD affected pregnancy (48,49), and data from clinical trials support the hypothesis that this apparent reduction in risk may be specifically attributable to folic acid (50,51), although the mechanisms underlying the protective effects of folic acid are not fully understood.

A potential link among fumonisins, folate deficiency, and increased risk for NTD seemed plausible based on the research findings of Stevens and Tang and colleagues (52,53), who demonstrated that receptor-mediated folate uptake was reduced by up to 50% in Caco-2 cells pretreated with fumonisin. The placental, high-affinity folate transporter [folate binding protein 1, Folbp1(murine); folate receptor α (human)] is a glycosylphosphatidylinositol (GPI)-anchored protein (54) associated with membrane microdomains (rafts) enriched in cholesterol and sphingolipids (55), which was previously shown to be critical for early embryonic development (56). Fumonisin affects the transporter by altering both its endocytic trafficking (53) and the amount of the receptor that is available for transport (Smith, E. R. and Stevens, V. L., unpublished data). These findings provided a conceptual framework (**Fig. 1**) whereby exposure to FB_1 might be a risk factor for NTD by disrupting folate utilization via depletion of cellular sphingolipids needed for normal receptor function.

Induction of developmental abnormalities in mouse models for neural tube defects

Despite this suggestive link between fumonisins and NTD, developmental defects were not reported in studies of rats (57), rabbits (33), and mice (58) exposed to FB_1 orally throughout the period of neural tube closure, and the dosages where developmental effects (fetal toxicity) were seen also produced maternal toxicity. FB_1 is toxic for developing chick embryos (59) as well as rat embryos in whole embryo culture

[4] NTD are common congenital malformations that occur when the embryonic neural tube, which ultimately forms the brain and spinal cord, fails to close properly during the first few weeks of development. Anencephaly results from failed closure of the anterior neural tube, with absence of the bones of the cranial vault and absent or rudimentary cerebral and cerebellar hemispheres and brainstem; spina bifida refers to incomplete development and fusion of one or more vertebral arches with associated involvement of the posterior neural tube. NTD are among the most common of all human birth defects, yet their etiologic basis and embryology remain poorly understood. Maternal dietary folate deficiency is a risk factor for neural tube defects (46); however, there is also a strong genetic susceptibility component, and other environmental factors have been suspected to exist.

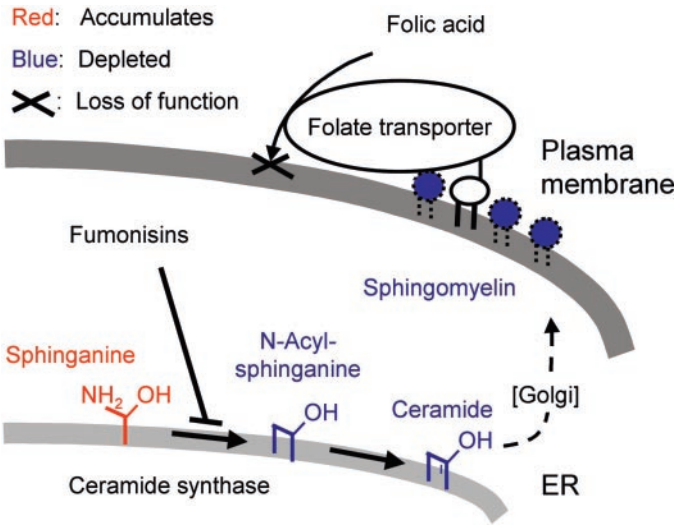

FIGURE 1 Disruption of sphingolipid metabolism and folate transport by fumonisins. The scheme shows the step where fumonisins inhibit sphingolipid biosynthesis (the acylation of sphinganine by ceramide synthase in the endoplasmic reticulum), thereby reducing the formation of sphingomyelin, which is a major component of the plasma membrane and is required for the proper function of GPI-anchored proteins, such as the folate transporter.

(60), but although there is a significant increase in NTD in the latter model, the abnormalities induced by FB_1 (and the aminopentol backbone AP_1) (61) occur in conjunction with retardation of overall growth and development. It is possible nonetheless that exposure to fumonisins might influence another risk factor, such as folate deficiency, to influence the susceptibility of animals to NTD.

Such an interaction was uncovered by studies (62) with early somite neurulating mouse embryos prepared for whole embryo culture on d 9 of gestation, which showed that FB_1 affects overall growth and causes cranial neural tube defects (**Fig. 2***A*, *top*) in a dose-dependent manner, and supplementing the medium with folate ameliorated these effects (Fig. 2*B*, *bottom*). The protection by folate is unlikely to be due to interference with the uptake of FB_1 because sphinganine was elevated in all FB_1-exposed embryos, irrespective of folate concentration. Therefore, fumonisins are able to inhibit embryonic sphingolipid metabolism and this appears to interfere with folate utilization to produce embryotoxicity and neural tube defects.

A relationship between maternal exposure to fumonisin and developmental abnormalities in the offspring was recently demonstrated in an in vivo mouse model (63–65). Pregnant dams from the highly inbred LMBc murine strain (66,67) were time-mated and injected intraperitoneally with 20 mg/kg body weight FB_1 on gestational d 7.5 and 8.5. Dams were killed on d 17.5 and the fetuses examined for NTD. In the FB_1 treated groups (n = 10 litters), 79% of the pups (78/99) presented with exencephaly [see Fig. 2C; exencephalic (*top*), normal (*bottom*)], whereas none of the pups in the control groups (n = 10 litters) was observed to have neural tube defects. Administration of 10 mg/kg body weight FB_1 produced NTD in 24% of the pups (n = 10 litters). The ability of supplemental maternal folate to protect against the teratogenic affects of FB_1 was also investigated. Pregnant LMBc dams were supplemented daily throughout gestation with 50 mg/kg body weight folinic acid (oral gavage) or 50 mg/kg body weight folic acid (intraperitoneal injection), beginning on gestational d 0.5. The incidence of NTD in FB_1 exposed litters following maternal folate supplementation was reduced from 79 to 43% (n = 10 litters). Since FB_1 may act via inhibition of the biosynthesis of sphingolipids that are required for the function of the folate receptor, and previous reports have shown colocalization of gangliosides with GPI-anchored membrane receptors (such as the folate receptor) (68–71), pregnant dams were administered ganglioside G_{M1} (10 mg/kg body weight per day, i.p.) the day before, during, and after fumonisin administration (GD 6.5–9.5). This strategy reduced the incidence of NTD to only 5% (n = 11 litters), which is a significant protective intervention.

Incidence of neural tube defects in countries with consumption of fumonisin-contaminated maize

If fumonisin consumption is a risk factor for NTD in humans, this association might be most evident among populations that consume the highest amounts of maize, such as those of Central and South America and portions of southern Africa and Asia. For example, adults in Guatemala regularly consume several hundred grams of maize daily in the form of tortillas. In 1995 a survey of tortilla consumption and fumonisin amounts was conducted in households in Santa Maria de Jesus (Sacatepequez) and Patzicia (Chimaltenango), which are typical rural Kaqchikel-speaking Mayan communities of Guatemala, located in the Central Highlands (72). Tortillas and nixtamal, the base-treated corn flour that is used to prepare masa for tortillas, was obtained from 50 households in each town and analyzed for the major fumonisin species, FB_1 and

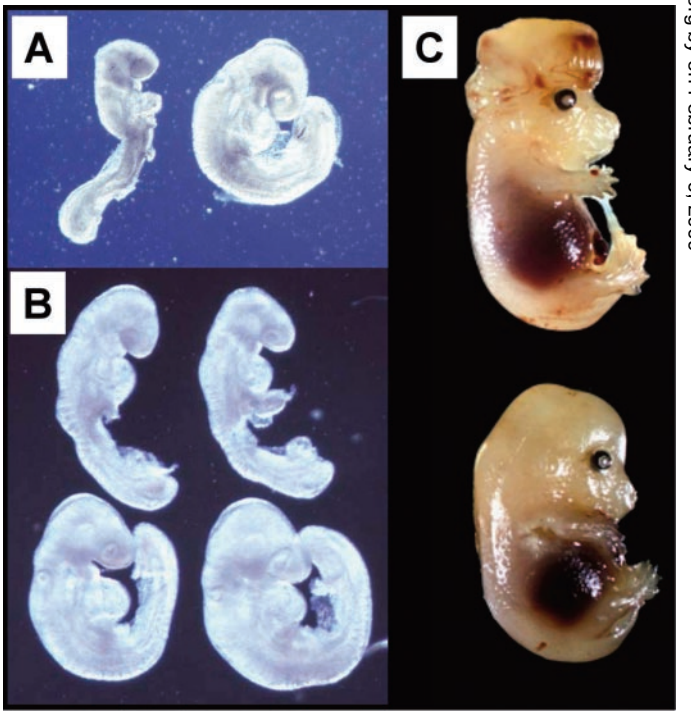

FIGURE 2 Effects of fumonisin B_1 (FB_1) in mouse embryonic development. *A*: Embryos after 24 h in culture medium containing none (right) or 50 μmol/L FB_1 (left); *B*: Embryos after 24 h in medium containing 50 μmol/L FB_1 (*top*) or 50 μmol/L FB_1 plus 1 μmol/L folinic acid (*bottom*) (*panel B* is reproduced from Fig. 3 of Ref. 62). *C*: Fetus from pregnant LMBc dam injected i.p. with 20 mg/kg body weight FB_1 on gestational d 7.5 and 8.5 and killed on d 17.5 (*top*) versus normal fetus at this gestational age (*bottom*).

AP_1. The latter is produced when the tricarballylic acids of FB_1 are base hydrolyzed and also inhibits ceramide synthase (73), although AP_1 is less toxic than FB_1 (32,74,75). The average fumonisin content (FB_1 + AP_1) of tortillas from Santa Maria de Jesus was 27 μg/g dry weight versus 8 μg/g from Patzicia.[5] A high percentage (66%) of the tortillas from Santa Maria de Jesus (and over one third from Patzicia) contained $\geq$10 μg FB_1 + AP_1/g dry weight. Follow-up studies found that fumonisin contamination is highly variable: for the years 2000 to 2002, only 6 to 8% of the maize samples had >3.7 μg of total fumonisins/g dry weight (Torres, O. R. and Riley, R. T., unpublished data). Fumonisins were also reported in Mexican tortillas (76).

Little is known about the incidence of NTD in rural areas of Guatemala; however, a recent retrospective study of the prevalence of children born with NTD (77) reviewed clinical files of live newborns in national and regional hospitals of different departments of Guatemala (Geographical and Administrative Divisions of the country) with the following inclusion criterion: living newborns of either gender presenting with neural tube defects during the year 2000. Some regions have strikingly high frequencies (compared to that for the general U.S. population of $\leq$3/10,000 live births), such as 106 NTD/10,000 live births in Quetzaltenango, which has a mostly indigenous population that consumes high amounts of maize as their staple food (**Fig. 3**). The most frequent defect was myelomeningocele.

In South Africa, high NTD incidences have been found in a rural Transkei district in the Eastern Cape Province (61/10,000) (78) and in rural areas in the Limpopo Province (35/10,000) (79); in contrast, far lower incidence figures are reported in urban regions such as Cape Town (1.06/10,000) (80), Pretoria (0.99/10,000) (81), and Johannesburg (1.18/10,000) (82). High incidence rates (57 to 73/10,000) are also reported in rural areas in the northern provinces of China (83,84). The inhabitants of both the Transkei region and the northern provinces of China are likely to be exposed periodically to high fumonisins levels as a result of the consumption of fungal contaminated maize (85,86).

Neural tube defects along the Texas-Mexico border

Elevated NTD rates have been noted along the Texas-Mexico border (ranging from 27/10,000 live births in 1990–1991 to 15/10,000 live births in a usual year) (41). It is not clear why the NTD rate along the border is 3 to 5 times that observed elsewhere in the United States (41). A number of possibilities have been evaluated in a recent study (Missmer, S. A., Suarez, L., Felkner, M., Wang, E., Merrill, A. H., Rothman, K. J., and Hendricks, K. A., unpublished data) from March 1995 through May 2000, in which 184 Mexican-American women with NTD-affected pregnancies and 225 women with healthy live births were identified from residents delivering or terminating pregnancies in hospitals or birthing centers in any of the 14 Texas-Mexico border counties. Case and control women were interviewed in person about medical, occupational, environmental, and dietary exposures during the periconceptional period and maternal blood samples were collected. In addition, locally made tortillas were collected throughout the region.

After adjusting for body mass index, date of conception, and serum vitamin B-12, intermediate intake of homemade tortillas during the first trimester (301 to 400 tortillas, compared to low, $\leq$100) was associated with increased odds of an NTD-affected pregnancy (odds ratio, OR = 2.4; 95% CI = 1.1–5.3) and the association was higher (OR = 2.9, CI = 1.4–5.9) for consumption of any homemade tortillas compared to store purchased tortillas. Although the odds ratio was slightly increased (OR = 1.6, 95% CI = 0.5–5.1) for women with a biomarker for fumonisin exposure (the serum sphinganine:sphingosine ratio, Sa:So), 0.31–0.35 compared to those with Sa:So < 0.10, the confidence interval included 1. Paradoxically, the highest Sa:So ratios (>0.35) were not associated with increased NTD (OR = 0.3, 95% CI = 0.1–1.2). A similar bell-shape relation was observed for self-reported absolute number of tortillas eaten during the first trimester of pregnancy and for absolute micrograms per kilogram of woman's weight per day of fumonisin exposure as estimated from the local tortilla samples. The observed bell-shape relation may reflect an increased prevalence of NTD at intermediate levels of exposure and an increase in fetal death at higher levels of exposure. Further analyses in humans are necessary to determine temporal associations and possible teratogenic thresholds.

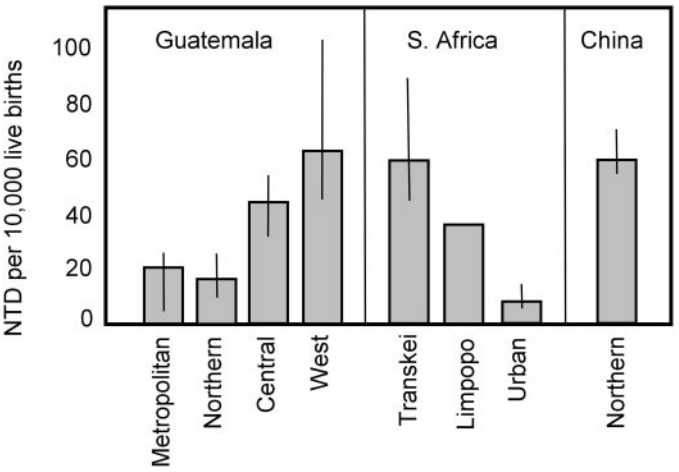

FIGURE 3 Incidence of neural tube defects (NTD per 10,000 live births) in different regions of Guatemala, South Africa, and China. Mean incidence and range in incidence of various locations within the regions or countries are shown; the bar for Limpopo represents one data point. Incidence rates for the United States are similar to those for urban areas in South Africa (41).

The higher association with homemade tortillas is intriguing. The majority of FB_1 (up to 80%) is removed during nixtamalization and the commercial manufacture of fried tortilla chips (87,88), but less is known about the fate of fumonisins in homemade tortillas. A study of some of the smaller facilities in Cameron County, Texas, found that the methods used were similar to those in commercial production (88) but varied in process details, such as the use of lower calcium hydroxide in some recipes (De La Campa, R., Miller, J. D. and Hendricks, K., unpublished data).

SUMMARY

In summary, we suggest that fumonisin consumption is a risk factor for human neural tube defects (and related birth defects such as craniofacial abnormalities), especially when there are other risk factors such as genetic susceptibility or limited availability of dietary folate. This hypothesis is based on current knowledge regarding the mechanism(s) of action of

[5] For comparison, if a 55 kg woman consumes 200 g of tortillas at 27 μg of fumonisins/g, this is equivalent to ca 100 μg of fumonisins/kg body weight.

fumonisins as inhibitors of sphingolipid biosynthesis (and thereby of folate transport), recent reports that fumonisins induce developmental abnormalities in mouse embryos in culture and LMBc mice in utero, and the incidence of NTD in regions of the world where substantial consumption of maize and fumonisins has been documented or plausibly suggested. It would be prudent to monitor this possibility.

ACKNOWLEDGMENTS

The authors are grateful to the numerous colleagues who have helped with the work described in this review and to Kate Merrill for help in preparing the manuscript.

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